AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 111:489–501 (2000)

Phenotypic Covariance Structure in Tamarins (Genus *Saguinus*): A Comparison of Variation Patterns Using Matrix Correlation and Common Principal Component Analysis

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KEY WORDS cranial morphology; Callitrichidae; variability; quantitative methods

ABSTRACT Constancy of variation/covariation structure among populations is frequently assumed in order to measure the differential selective forces which have caused population differentiation through evolutionary time. Following Steppan ([1997] Evolution *51:*571–594), this assumption is examined among closely related tamarin species (genus *Saguinus*), using two distinct approaches applied to the task of evaluating similarity in patterns of morphological variation: common principal component analysis and matrix correlations. While the results of these analyses may appear contradictory, closer examination reveals them as complementary, highlighting the wisdom of combined methodologies. Overall, the results reveal a close relationship among the morphologically based variance structures of the tamarin species a relationship whose pattern is consistent with the pattern of phylogenetic relatedness as found via a molecular genetic study. More specifically, both methodological approaches provide some support for divergence of *S. geoffroyi* and *S. oedipus* (with regards to their patterns of morphological variation) from other tamarin species. This suggests that variance/covariance structure may have diverged through evolutionary time in the tamarin lineage, placing assumptions of constancy in doubt. Am J Phys Anthropol 111: 489–501, 2000.

The pattern and magnitude of intrapopulation variation contribute to our understanding of evolution and the degree of similarity between related organisms. Similarly, our understanding of this variation (and its boundaries or limits) shapes the way we divide and understand our world and the organisms within it (in terms of their distribution through both space and time). Among one group of New World monkeys, the tamarins (family Callitrichidae, genus *Saguinus*; von Hoffmannsegg, 1807), analyses into variation in coat color, body size, and craniofacial morphology have all been applied to the task of understanding relationships among populations (Hershkovitz, 1977; Ferrari, 1993a,b; Cheverud, 1995, 1996). Each of these studies focuses on phenotypic variation; as the target of selection, the phenotype offers unique insight into evolutionary processes, and can be used to address evolutionary questions utilizing fossils or large museum collections, where quantitative genetic data are not available. Additionally, phenotypic variation and covariation are often used as a surrogate for quantitative genetic variation/covariation,

Grant sponsor: National Science Foundation; Grant number: SBR-9632163.

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Received 22 June 1999; accepted 7 December 1999.

since large samples of quantitative genetic data are only obtainable via controlled captive breeding programs. This substitution is viable, because phenotypic covariance matrices for morphological traits reflect underlying genetic organization and mechanisms, and are therefore generally similar to genetic covariance matrices (Cheverud, 1988; Arnold, 1992; Roff, 1995, 1996; Koots and Gibson, 1996).

This substitution is also effective for understanding both micro- and macroevolutionary patterns, since patterns of genetic variation and covariation play an important role in determining the average response to selection (Lande, 1979). This is illustrated by the equation $\Delta z = G\beta$, where Δz is the $n \times 1$ vector of change in trait means, β is the $n \times 1$ selection gradient vector measuring the selection acting on the n traits, and G is the $n \times n$ additive genetic variance/covariance matrix (Lande, 1979). When the structure of genetic variation remains constant over generations, it is possible to measure the differential selection operating on two divergent populations or species by rearranging the equation to yield $\beta = G^{-1}\Delta z$, where Δz is the observed difference between species means and β is the selection responsible for the phenotypic separation of the species (Lande, 1979). The ability to interpret this equation depends on whether constancy of variation structure is a valid assumption, i.e., whether patterns of covariation behave predictably over macroevolutionary time (Steppan, 1997a,b; Lande, 1979; Price et al., 1984; Cheetham et al., 1993).

Following Steppan (1997a), this assumption of constancy of variation is examined among closely related tamarin species using two distinct approaches: matrix correlation and common principal component analysis. Phenotypic covariation in cranial morphology is substituted for genetic covariation, and therefore the constancy of the covariation in cranial morphology is at issue. In his analysis of species and subspecies of leaf-eared mice, Steppan (1997a) compared these two approaches, and found that while the phenotypic covariance structure was not constant, divergence between populations was small and not associated with their phylogenetic pattern. There is some evidence that subspecific populations of tamarins (represented by the single species *Saguinus fuscicollis*) show a similar phenomenon; divergence in variation structure is random and of small magnitude (Cheverud, 1995). In order to determine whether constancy of covariation structure exists at a higher taxonomic level (species) among tamarins, patterns of morphological variation and covariation are compared among six species: *Saguinus fuscicollis*, *S. geoffroyi*, *S. midas*, *S. mystax*, *S. nigricollis*, and *S. oedipus*.

MATERIALS AND METHODS

Measurements were obtained from a total of 848 crania of adult tamarins. Crania with fused spheno-occipital and spheno-ethmoidal sutures were considered adult. The *Saguinus* specimens were obtained from collections at the American Museum of Natural History (AMNH, New York), the British Museum of Natural History (BMNH, London), the Field Museum of Natural History (FMNH, Chicago), the National Museum of Natural History (NMNH, Washington, DC), the University of Tennessee (ORAU, derived from the Marmoset Research Center, Oak Ridge Associated Universities' colony), and the University of Sao Paolo (USP, Brazil), as follows: *Saguinus fuscicollis* (n = 289; AMNH, BMNH, FMNH, NMNH, ORAU, USP), *S. geoffroyi* (n = 132; AMNH, FMNH, NMNH), *S. midas* (n = 116; AMNH, BMNH, FMNH, NMNH), *S. mystax* (n = 72; AMNH, BMNH, FMNH, NMNH, ORAU, USP), *S. nigricollis* (n = 59; AMNH, BMNH, NMNH, ORAU), and *S. oedipus* (n = 180; AMNH, NMNH, ORAU).

Three-dimensional coordinates were recorded for 36 landmarks using a Polhemus 3Space digitizer (Table 1, Fig. 1). Each specimen was digitized twice to minimize measurement error (see Cheverud, 1995); the average of repeated measures was used for further analyses. A set of 39 linear measurements, averaged between left and right sides and chosen to describe cranial morphology without excessive redundancy, was calculated from the coordinate values (Table 2). Phenotypic correlation matrices and variance/covariance matrices were obtained

TABLE 1. Craniofacial landmarks recorded from tamarin crania, using three-dimensional digitizer[1]

Landmark	Description	Position(s)
IS	Intradentale superior, A	Midline
PM	Premaxillary suture at the alveolus, A	Right, left
NSL	Nasale, A	Midline
NA	Nasion, A	Midline
BR	Bregma, AP	Midline
PT	Pterion, AP	Right, left
FM	Fronto-malare, A	Right, left
ZS	Zygomaxillare superior, A	Right, left
ZI	Zygomaxillare inferior, A	Right, left
MT	Maxillary tuberosity, A	Right, left
PNS	Posterior nasal spine, A	Midline
APET	Anterior petrous temporal, A	Midline
BA	Basion, AP	Midline
OPI	Opisthion, AP	Midline
EAM	Anterior external auditory meatus, A	Right, left
PEAM	Posterior external auditory meatus, A	Right, left
ZYGO	Inferior zygo-temporal suture, A	Right, left
TSP	Temporo-spheno-parietal junction, A	Right, left
TS	Temporo-sphenoidal junction at petrous, AP	Right, left
JP	Jugular process, AP	Right, left
LD	Lambda, P	Midline
AS	Asterion, P	Right, left

[1] A (anterior) or P (posterior) after the landmark description indicates in which position(s) the landmark was recorded. Landmarks are also identified in Figure 1. Adopted from Cheverud (1995).

for these 39 cranial and facial variables in six species of tamarins, using the residual correlation matrix and residual covariance matrix from a MANOVA with the 39 traits as dependent variables and subspecific affiliation as the independent variable, thus pooling the correlations and covariances across subspecies. For *S. oedipus*, the Oakridge and non-Oakridge groups were similarly pooled, since differences between the two groups are significant for a number of variables.

Analysis 1: common principal components model

While most statistical tests of matrix similarity consider a null hypothesis of no structural similarity, the common principal components (CPC) analysis tests whether matrices share more complex relationships (Flury, 1988; Steppan, 1997a; Phillips and Arnold, 1999; Arnold and Phillips, 1999). For example, two matrices might be proportional but not equal, or might share principal component structure (eigenvectors) while differing in the eigenvalues associated with the components. Similarly, the two matrices might share some portion of their principal components: the partial common principal components (PCPC) model. The common principal component approach (CPC) tests each of these hypotheses, by building up each level in the hierarchy, from unrelatedness to equality, and testing the significance of each level against the next lower level (Steppan, 1997a; Phillips and Arnold, 1999; Arnold and Phillips, 1999). This hierarchy is based on the realization that if two matrices share three principal components in common, they necessarily share two in common. Similarly, if two matrices are equal, they are necessarily proportional, and satisfy the CPC and all PCPC models. Common principal component analyses were conducted using the program CPC (Phillips, 1998), which performs the analyses outlined by Flury (1988). The number of partial common principal components analyzed was limited to seven, since a standard t-test shows that beyond the fifth principal component, few loadings are significantly different from zero for this data set (the choice of seven PCs was therefore conservative).

Following these analyses, the eigenstructures of the tamarin species were further examined, in order to assist in interpreting the CPC results. Correlations were calculated between all pairwise comparisons of the first seven standardized principal components for all species comparisons. Additionally, each value of the standardized PC(1) for each species was divided by $(1/\sqrt{39})$ to assess morphological divergence from isometry (Jolicoeur, 1963).

Analysis 2: matrix correlation model

Elementwise matrix correlations were calculated between the correlation matrices of all possible pairs of species. In order to estimate the impact of sampling error, matrix repeatability (Cheverud, 1996) was determined. This technique compares observed correlations to a theoretical maximum, in order to find the actual (adjusted)

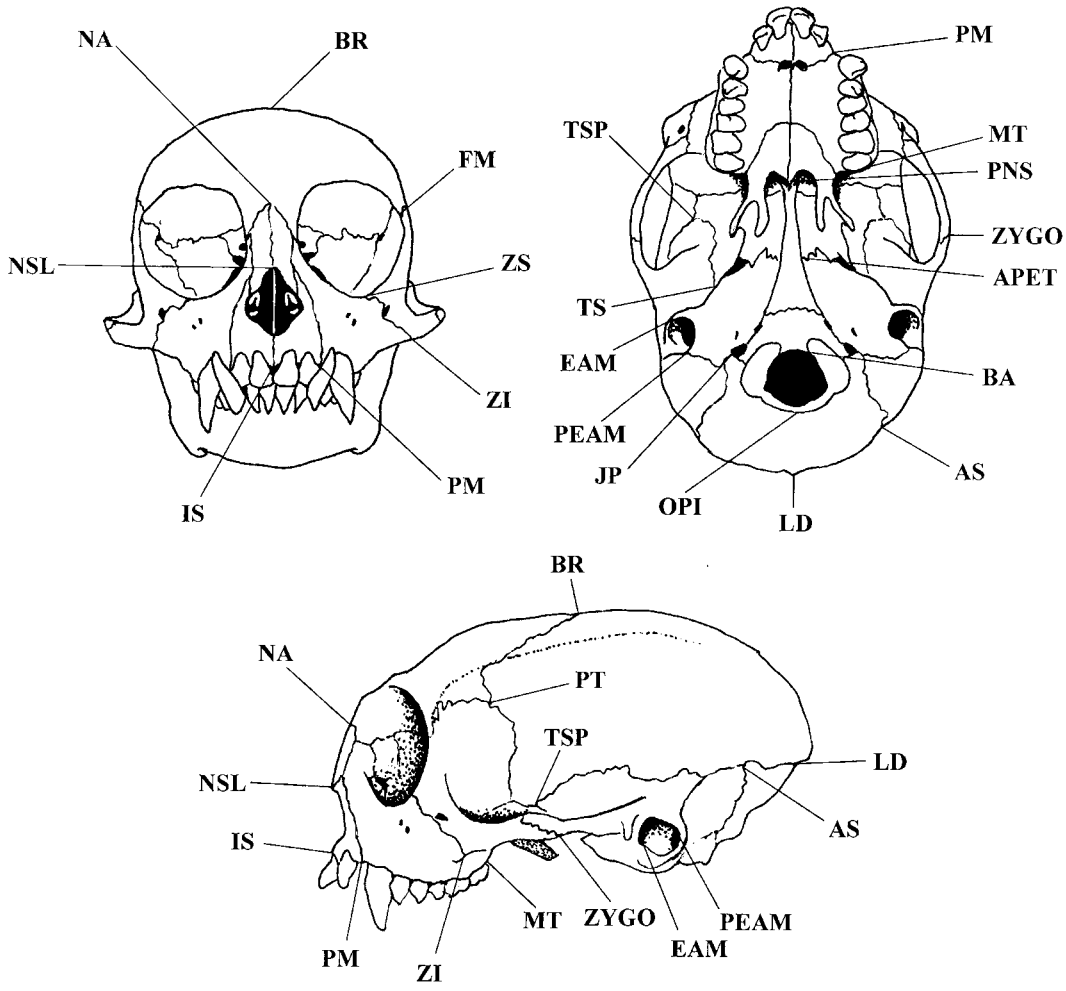

Fig. 1. Craniofacial landmarks recorded from tamarin crania using three-dimensional digitizer. Refer to Table 1 for descriptions of landmarks.

matrix correlation (accounting for error). The observed variance of matrix elements (V_{obs}) is composed of the error variance of the elements (V_{err}) and the variance of the true population values (V_t):

$$V_{obs} = V_t + V_{err}.$$

Solved differently, $V_t = V_{obs} - V_{err}$, where V_{err} is the squared standard error of the average correlation in the matrix, and V_t is the actual variance of matrix elements. The theoretical maximum matrix correlation between two matrices is then $R_{max} = \sqrt{t_1 t_2}$, with t_1 equal to the actual variance divided by the observed variance (V_t/V_{obs}) for the first species' matrix, and t_2 equal to (V_t/V_{obs}) for the second species' matrix. Adjusted matrix correlations are the observed correlation (R_{obs}) between the two matrices divided by the maximum matrix correlation (R_{max}) calculated for that pairwise comparison.

Additionally, hypotheses of morphological integration within each tamarin species were tested. Morphological integration, in its most general sense, refers to the connections or relationships among morphological elements. As described previously (Cheverud, 1995, 1996), matrices are used to as-

TABLE 2. Thirty-nine linear craniofacial measurements and their membership in functional/developmental groups[1]

Measurement	Functional/developmental group(s)
IS-PM	Oral
IS-NSL	Nasal
IS-PNS	Oral, nasal
PM-ZS	Oral
PM-ZI	Oral
PM-MT	Oral
NSL-NA	Nasal
NSL-ZS	Nasal
NSL-ZI	Oral, nasal
NA-BR	Cranial vault
NA-FM	Orbit
NA-PNS	Nasal
BR-PT	Cranial vault
BR-APET	Cranial vault
PT-FM	Orbit
PT-APET	Cranial vault
PT-BA	Cranial vault
PT-EAM	Cranial vault
PT-ZYGO	Zygomatic
PT-TSP	Cranial vault, zygomatic
FM-ZS	Orbit
FM-MT	Zygomatic
ZS-ZI	Oral
ZI-MT	Oral
ZI-ZYGO	Zygomatic
ZI-TSP	Zygomatic
MT-PNS	Oral
PNS-APET	Cranial base
APET-BA	Cranial base
APET-TS	Cranial base
BA-EAM	Cranial base
EAM-ZYGO	Zygomatic
ZYGO-TSP	Zygomatic
LD-AS	Cranial vault
BR-LD	Cranial vault
OPI-LD	Cranial vault
PT-AS	Cranial vault
JP-AS	Cranial base
BA-OPI	Cranial base

[1] Adapted from Cheverud (1995). Refer to Table 1 for description of landmarks used in measurements.

sess morphological integration within six virtually independent regions of the cranium (cranial vault, cranial base, orbit, oral, zygomatic, and nasal; see Table 2) and within the cranium as a whole (total morphological integration), by calculating elementwise matrix correlations between the observed species' correlation matrices and morphological integration connectivity matrices. These connectivity matrices are constructed in the following manner: when two traits belong to the specified developmental set, a value of one is entered in the developmental matrix; otherwise, a value of zero is entered. For the total integration matrix, a one is entered if traits belong to the same developmental trait set, and a zero is entered otherwise (Cheverud, 1995, 1996).

The statistical significance of all of the elementwise matrix correlations was obtained using quadratic assignment procedures, sometimes referred to as Mantel's test (Cheverud et al., 1989). This procedure is performed by randomly permuting the rows and associated columns of one matrix and then calculating the matrix correlation between the unaltered matrix and the permuted matrix. This permutation was repeated 10,000 times, and a distribution of matrix correlations expected under the null hypothesis of no structural similarity between the matrices was obtained. The observed correlation was then compared to the empirically derived distribution, and the proportion of permutation correlations greater than or equal to the observed correlation functioned as an estimate of the probability of obtaining the observed correlation, given that the null hypothesis is true.

Additionally, a discriminant function analysis was performed and the canonical scores of the group means were used to calculate the morphological distances (D^2) between species, to see whether variation in covariance among tamarin species corresponds to the distances between population means. The D^2 matrices were correlated in turn with the correlations between the within-species correlation matrices and with the pairwise correlations of the first principal components of the V/CV matrices. Each of these measures of variation was then compared to the results of the CPC analysis, and interpreted in the context of known phylogenetic relationships between the species as measured by phylogenetic analysis (see Cropp et al., 1999) to examine how closely measurements of morphological similarity reflect phylogenetic relationship. Cropp et al. (1999) generated their phylogenetic tree by manually aligning homologous sites of the mtDNA sequences and analyzing them with phylogenetic analysis using parsimony (PAUP). The outgroup reference taxa consisted of *Callimico*, *Callithrix*, *Cebuella*, and *Leontopithecus*, and the most parsimonius phylogenetic reconstruction for the genus *Saguinus* was obtained using the

TABLE 3. Comparison of covariance matrices among all six tamarin species[1]

Hierarchy		Morphology estimate (parametric)			
Higher	Lower	χ^2	*df*	*P*	AIC
Equality	Proportionality	290.31	5	0.0001	9,231.5
Proportionality	Full CPC	1,347.55	190	0.0001	8,951.2
Full CPC	CPC(7)	4,922.72	2,480	0.0001	7,983.6
CPC(7)	CPC(6)	289.08	160	0.0001	8,020.9
CPC(6)	CPC(5)	523.63	165	0.0001	8,051.8
CPC(5)	CPC(4)	326.20	170	0.0001	7,858.2
CPC(4)	CPC(3)	408.13	175	0.0001	7,872.0
CPC(3)	CPC(2)	349.23	180	0.0001	7,813.9
CPC(2)	CPC(1)	275.16	185	0.0001	7,824.7
CPC(1)	Unrelated	499.50	190	0.0001	7,919.5
Unrelated					

[1] At each step in the hierarchy, the hypothesis labeled "Higher" is tested against the hypothesis on the step below, "Lower." Estimates of the genetic matrix are based on the variance/covariance of the cranial morphology (with parametric evaluation of sampling properties). On an objective hypothesis-testing basis, there is no equality, proportionality, or shared principal component structure among the six V/CV matrices. However, the best solution under the model-building approach may be indicated by the minimum value of Akaike information criterion (AIC), namely, CPC(3) for this comparison.

heuristic search option (for further details, see Cropp et al., 1999).

RESULTS

On a purely "objective" hypothesis-testing basis, the results of the CPC analysis say that there is no equality, proportionality, or shared principal component structure among any of the V/CV matrices, whether analyzed together as a group, or in a pairwise fashion. In other words, the tamarin covariance matrices are not significantly similar in the structure of either their eigenvalues or eigenvectors. This is illustrated by the fact that you cannot take the first step up from unrelated structure to CPC(1) without encountering a significant *P*-value in each of the analyses (i.e., the hypothesis of a single common principal component, CPC(1), is rejected; see Table 3 for an example). However, Flury (1988) argues that a test of best fit is more appropriate than a test of fit or lack of fit based on the standardized rejection criteria (see also Phillips and Arnold, 1999; Arnold and Phillips, 1999). Although a hypothesis-testing approach may be more appropriate for this study, we also examined the results of the model-fitting approach to see if they differ substantially. Under a model-fitting approach, the model with the lowest Akaike information criterion (AIC), a statistic which balances the size of the log-likelihood function with the number of parameters estimated (see Steppan 1997a), is considered the best-fitting model (given the total amount of information available for testing this model). The AIC criterion suggests that shared CPC structure (to varied degrees) is the best fit for each of the pairwise comparisons (Table 4); most of the species have similar overall patterns of covariance/variation as shown by their low AIC value for their entire principal component structure (full CPC). However, *S. oedipus* and *S. geoffroyi* may be more similar to each other than they are to some of the other species (such as *S. fuscicollis*), with whom they only share partial CPC structure; there is also partial CPC structural similarity between *S. nigricollis* and *S. fuscicollis*. However, the inability to test the significance of these AIC results, combined with their contradiction of the hypothesis-testing based results, presents something of a dilemma.

To resolve this dilemma, further evaluation of the eigenstructure is warranted. Examining the eigenstructure of the covariance matrices reveals very different results. The first seven standardized eigenvectors of each species were correlated with the eigenvectors of the other species in a pairwise fashion. The magnitude of correlation for each pairwise species comparison varies, and while there is definitely similarity between the species, it is not always clearly interpretable. Correlations between the first principal components for each species are high (excepting *S. geoffroyi*), indicating similarity in allometric scaling, although

TABLE 4. Best AIC solutions for comparison of covariance matrices between each paired tamarin species[1]

	S. fuscicollis	*S. nigricollis*	*S. midas*	*S. mystax*	*S. geoffroyi*	*S. oedipus*
S. fuscicollis	*Equality*					
S. nigricollis	*CPC(6)*	*Equality*				
S. midas	*Full CPC*	*Full CPC*	*Equality*			
S. mystax	*Full CPC*		*Full CPC*	*Equality*		
S. geoffroyi	*CPC(4)*	*Full CPC*	*Full CPC*	*Full CPC*	*Equality*	
S. oedipus	*CPC(6)*	*CPC(7)*	*Full CPC*	*Full CPC*	*Full CPC*	*Equality*

[1] Based on Flury's hierarchy of tests (V0.95), step-up procedure (Flury, 1988; Phillips, 1998; Phillips and Arnold, 1999; Arnold and Phillips, 1999). The best solution under the model-building approach is indicated by the minimum value of the Akaike information criterion (AIC), and is shown for each comparison. The comparison between *S. mystax* and *S. nigricollis* was not possible for this set of variables (the matrices were "ill-formed," due to high levels of dissimilarity in their relative eigenstructures).

TABLE 5. Correlations between the standardized first principal components (PC1) of the V/CV matrices for each species

	S. fuscicollis	*S. nigricollis*	*S. midas*	*S. mystax*	*S. geoffroyi*	*S. oedipus*
S. fuscicollis	*1.00*					
S. nigricollis	*0.95*	*1.00*				
S. midas	*0.97*	*0.90*	*1.00*			
S. mystax	*0.83*	*0.79*	*0.82*	*1.00*		
S. geoffroyi	*0.65*	*0.60*	*0.65*	*0.85*	*1.00*	
S. oedipus	*0.93*	*0.87*	*0.94*	*0.90*	*0.74*	*1.00*

the magnitude of this relationship varies depending on the species comparison, with the correlation between PC(1)s ranging from $r = 0.60-0.97$ (Tables 5, 6). This reveals a degree of similarity not detectable in the formal results of the CPC analysis, which simply indicates statistically significant divergence between the first principal components of the matrices. Additionally, there is some suggestion that further eigenvector structure is shared among species, since a number of correlation values between the first seven principal components are outside of the range $-0.4 < r < 0.4$ (a range of correlations which commonly occurs among 39-element vectors by chance alone; Cheverud et al., 1983). When the first principal component is converted to identify isometry, allometric similarities in covariance structure between tamarin species are apparent (Table 6). Again, *S. geoffroyi* stands out from the other species; the analyzed morphological traits vary uniquely when compared to the other tamarin species, as can be seen in their allometry vectors (Table 6). Large differences between the covariance structure of *S. geoffroyi* and the other species occur in the region of the midcrania, with negative values indicating relatively shortened cranial height (BR-APET), reduced zygomatic flaring (ZI-TSP), and some marked reduction in other variables of the zygomatic, oral, and nasal regions (ZYGO-TSP, EAM-ZYGO, MT-PNS, NA-FM). In fact, the overall pattern of the allometry vector differs in *S. geoffroyi* compared to the other tamarin species, suggesting that there is either a real difference in covariance pattern, or that the first principal component of *S. geoffroyi* may not measure the same morphological phenomena seen within the other populations.

Matrix correlation analysis reveals a similar phenomenon to that seen in the results of the allometry examination (Table 7), with elementwise matrix correlations between the correlation matrices of each species found to be significant, though variable. As expected, the matrix of correlations (MxC) between the species correlation matrices corresponds with, and is correlated with, that found when comparing the first principal component for each species ($r = 0.83$, adjusted; $P < 0.001$). In both cases, *S. fuscicollis* is most strongly correlated with *S. midas* and *S. nigricollis*, and less correlated with *S. oedipus*, *S. mystax*, and *S. geoffroyi*, in descending order. This pattern of relationship is maintained throughout both the MxC and PC(1) between species comparisons; generally, *S. fuscicollis*, *S. midas*, and *S. nigricollis* cluster closely together, *S. my-*

TABLE 6. Allometry vectors of covariance matrices[1]

	S. fuscicollis	*S. nigricollis*	*S. midas*	*S. mystax*	*S. geoffroyi*	*S. oedipus*
IS-PM	0.384	0.390	0.426	0.704	0.598	0.465
IS-NA	0.604	0.820	0.532	0.434	0.212	0.632
IS-PNS	1.421	1.695	1.079	1.874	2.526	1.481
PM-ZS	0.431	0.273	0.392	1.242	0.948	0.742
PM-ZI	0.982	0.918	0.272	1.190	1.544	1.441
PM-MT	0.812	0.656	0.688	1.174	0.656	1.063
NSL-NA	0.112	−0.013	0.112	0.138	−0.075	−0.121
NSL-ZS	0.745	0.564	0.716	1.511	1.027	1.169
NSL-ZI	1.522	1.407	1.522	1.417	1.383	1.874
NA-BR	0.940	0.732	1.249	1.603	1.364	1.694
NA-FM	0.547	0.976	0.694	1.773	1.474	0.980
NA-PNS	1.424	1.503	1.540	1.413	1.134	1.248
BR-PT	0.289	0.201	1.088	1.430	1.129	1.206
BR-APET	0.716	0.642	1.144	0.118	−1.016	1.032
PT-FM	0.142	0.106	−0.064	0.183	−0.040	0.447
PT-APET	1.403	0.838	1.562	1.539	1.292	1.407
PT-BA	2.098	1.539	2.275	1.673	1.695	1.997
PT-EAM	1.770	1.609	1.898	0.624	0.735	1.612
PT-ZYGO	1.825	1.484	1.983	0.466	0.262	1.554
PT-TSP	1.109	0.962	1.270	0.461	0.283	1.012
FM-ZS	0.309	0.866	0.208	0.183	0.125	−0.135
FM-MT	0.950	1.144	1.094	0.834	0.558	0.838
ZS-ZI	0.938	0.449	0.651	0.275	0.661	0.799
ZI-MT	0.160	0.536	−0.123	0.656	0.176	0.588
ZI-ZYGO	0.645	1.446	1.107	0.329	0.595	0.721
ZI-TSP	0.562	0.931	0.690	−0.086	−0.954	0.012
MT-PNS	0.686	0.583	0.637	0.928	0.750	0.252
PNS-APET	−0.022	0.156	0.331	−0.034	−0.311	0.503
APET-BA	0.728	0.577	0.599	0.352	0.435	0.564
APET-TS	0.386	0.264	0.401	0.279	0.545	0.230
BA-EAM	1.222	0.914	0.995	1.190	1.230	0.792
EAM-ZYGO	0.143	−0.052	0.378	0.200	−0.492	0.141
ZYGO-TSP	0.855	0.680	0.878	0.090	−0.298	0.676
LD-AS	0.476	0.659	0.608	1.611	1.625	0.721
BR-LD	1.391	2.394	0.410	0.626	−0.454	0.801
OPI-LD	0.792	0.929	1.126	0.586	1.130	0.745
PT-AS	2.091	1.860	1.759	1.524	0.248	1.130
JP-AS	0.787	0.724	0.491	0.899	0.565	0.596
BA-OPI	0.263	0.269	0.164	0.263	−0.059	0.256

[1] The standardized first principal component for each species has been converted (divided by $(1/\sqrt{39})$) to identify regions where the variables diverge from isometry; values which are greater or less than 1.0 are positively or negatively allometric, respectively. Refer to Table 1 for descriptions of landmarks used in the measurements.

TABLE 7. Correlations between correlation matrices for each pairwise tamarin species comparison[1]

	S. fuscicollis	*S. nigricollis*	*S. midas*	*S. mystax*	*S. geoffroyi*	*S. oedipus*
S. fuscicollis	**0.90**	0.88	0.88	0.74	0.56	0.77
S. nigricollis	0.66	**0.63**	0.69	0.70	0.49	0.66
S. midas	0.74	0.57	**0.78**	0.69	0.61	0.73
S. mystax	0.62	0.49	0.53	**0.77**	0.87	0.77
S. geoffroyi	0.51	0.37	0.52	0.72	**0.91**	0.71
S. oedipus	0.66	0.47	0.58	0.61	0.61	**0.81**

[1] The matrix displays three sets of data: raw matrix correlations in the lower left half of the matrix, adjusted correlations in the upper right half of the matrix, and matrix repeatabilities (in bold) on the diagonal. Each calculation is based on the population correlation matrices, and is significant at $P = 0.0001$.

stax and *S. geoffroyi* cluster together, and *S. oedipus* is somewhere between these two clusters in terms of covariance patterns. The covariance/variation patterns of the species (in the form of adjusted MxC and PC(1)) are compared to morphological distances (D^2) between the species means (Table 8); the results are mildly correlated (PC(1) and D^2: $r = -0.43$, $P = 0.086$; MxC and D^2: $r = -0.50$; $P = 0.042$; see Fig. 2). While it is difficult to assign meaning to these values, certainly the similarity in pat-

TABLE 8. Morphological distances (D^2) between the means of all paired tamarin species

	S. fuscicollis	*S. nigricollis*	*S. midas*	*S. mystax*	*S. geoffroyi*	*S. oedipus*
S. fuscicollis	*0.0*					
S. nigricollis	*9.2*	*0.0*				
S. midas	*26.7*	*21.5*	*0.0*			
S. mystax	*30.5*	*29.9*	*31.3*	*0.0*		
S. geoffroyi	*63.3*	*59.0*	*61.0*	*51.6*	*0.0*	
S. oedipus	*57.2*	*51.5*	*54.7*	*55.4*	*26.5*	*0.0*

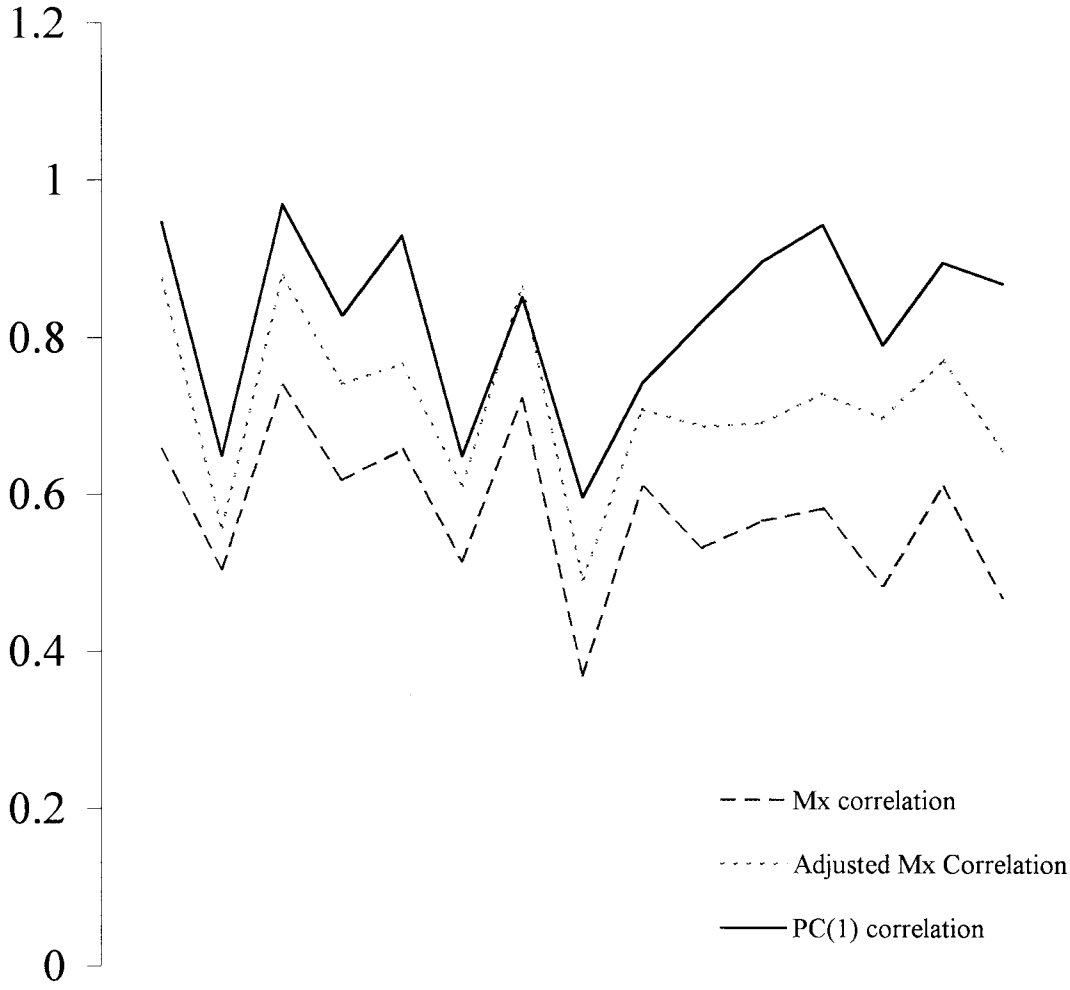

Fig. 2. Comparison of the three different methods used to compare tamarin species. Note that the correlation values (Y-axis) for each pairwise species comparison (X-axis) show a similar overall pattern, regardless of which method is used.

terning of the relationships is striking, and will be further interpreted in light of the tamarin phylogenetic relationships found by Cropp et al. (1999).

All six tamarin species fit the models of total morphological integration proposed by Cheverud (1995), with primary contributions from the higher correlations among

TABLE 9. Morphological integration for each tamarin species, displayed as the correlation between the correlation matrices for each species and the morphological integration matrices

	Total morphological integration	Cranial base	Oral	Nasal	Cranial vault	Zygomatic	Orbit
S. fuscicollis	*0.206***	*−0.076**	*0.203***	*0.014*	*0.198***	*−0.010*	*−0.085***
S. nigricollis	*0.133***	*−0.044*	*0.234***	*−0.006*	*0.041*	*0.000*	*−0.046*
S. midas	*0.189***	*−0.425*	*0.079*	*0.010*	*0.217***	*0.002*	*−0.063**
S. mystax	*0.153***	*−0.069*	*0.200***	*0.007*	*0.150***	*−0.071**	*−0.067***
S. geoffroyi	*0.130***	*−0.068**	*0.066*	*−0.002*	*0.166***	*0.031*	*−0.034*
S. oedipus	*0.184***	*−0.015*	*0.195***	*0.055*	*0.128***	*−0.025*	*−0.089****

* Significant at $P < 0.10$.
** Significant at $P < 0.05$.
*** Significant at $P < 0.01$.

traits in the oral region and cranial vault. This is consistent with the results of previous analyses of *S. fuscicollis* and *S. oedipus* (Cheverud, 1995, 1996), and suggests that a consistent pattern of total morphological integration within the cranium may exist more broadly among members of the genus *Saguinus*.

DISCUSSION

Two general conclusions can be drawn from this analysis. The first stems from the strict hypothesis testing approach of the CPC analysis, and states that the six species of tamarins vary uniquely; their patterns of variation and covariation are not equal, proportional, or similar in their eigenstructure. The second, supported by the matrix correlation results and the AIC interpretation of the CPC analysis, states that variation between species *is* structurally similar, particularly between the first principal components of the V/CV matrices. Based on the CPC analysis alone, the hypothesis of constant covariance matrices must be rejected. However, there are significant correlations and shared principal component structures among the covariance matrices when other methods of analysis are used. Which methods tell the "correct" story?

Steppan (1997) compares the results of CPC and correlation approaches, providing a discussion of the relative merits of the two methods. To summarize, matrix correlations provide an easily interpretable, continuously distributed statistic, which describes overall similarity, while CPC does not; however, unlike CPC, they do not have a direct statistical test for matrix identity (Steppan, 1997a). Small sample sizes can reduce matrix correlations, while they tend to cause acceptance of a higher degree of shared covariance structure with CPC analysis. Additionally, the accuracy of matrix correlations increases with increasing numbers of characters, while the likelihood of incorrectly rejecting common structure increases when using CPC with an increasing number of characters (Steppan, 1997a).

So while the varied results of this study are contradictory, they are not necessarily mutually exclusive when viewed with an eye towards Steppan's (1997a) conclusions. The CPC analysis results can be significant without being overly meaningful; it is possible that when the CPC test is applied to the task of evaluating data sets with large numbers of variables, such as this tamarin data set, highly significant heterogeneity can be found when there is only a small amount of difference between two populations. This is merely a function of degrees of freedom, and while it does not invalidate the Flury test, it does suggest that it is prudent to perform it in conjunction with other tests of similarity or difference in order to better answer questions such as, "Yes, they are different, but precisely how different?" or "Is this difference meaningful?"

With this in mind, the results open to new interpretation. The tamarin species are undoubtedly different, and each has its own unique structure of variation and covariation, as shown by the CPC analysis. Yet they are also remarkably similar, with moderate to high correlations between each

pairwise species comparison of their V/CV matrices. This measure of overall similarity can be further dissected to show that they are most similar in the structure of their first principal component, that patterns of correlation within each species are themselves highly correlated, and that general patterns of morphological integration are consistent between the species.

Evidence from molecular studies (Cropp et al., 1999) and analysis of feeding adaptations (Garber, 1992) supports the division of *Saguinus* into two major clades, with *S. fuscicollis* and *S. nigricollis* in one clade, and the remaining species in a second. The patterning of morphological similarity, shown by the results of the matrix correlations (MxC), corresponds somewhat with the molecularly derived phylogenetic relationships based on the molecular mtDNA evidence of Cropp et al. (1999) (see Fig. 3). *S. fuscicollis* and *S. nigricollis*, both members of the small-bodied clade of Cropp et al. (1999), are consistently closely associated via morphological variation. Cropp et al. (1999) place *S. midas* and *S. mystax* within the large-bodied clade with *S. oedipus* and *S. geoffroyi*. While the morphological evidence shown here also supports a close relationship between *S. oedipus*, *S. geoffroyi*, and *S. mystax*, in the morphological analyses (MxC), *S. midas* is most similar to *S. fuscicollis*, supporting a *fuscicollis/nigricollis/midas* grouping. This general pattern is reinforced through further examination of the eigenstructure of the first principal component, and corresponds with the morphological distances (D^2) obtained between the species means. So, despite the evidence that *S. midas* is not phylogenetically closely related to the small-bodied tamarins, it does have a pattern of morphological variation which is similar to that found in the small-bodied tamarins—and, indeed, it does look like them. These results suggest that both cranial morphology and, to some extent, patterns of morphological variation, may have been reorganized during the diversification of the large-bodied clade.

Overall, matrix correlation results are supported by closer examination of eigenstructure and by measures of morphological distances. Additionally, matrix correlation

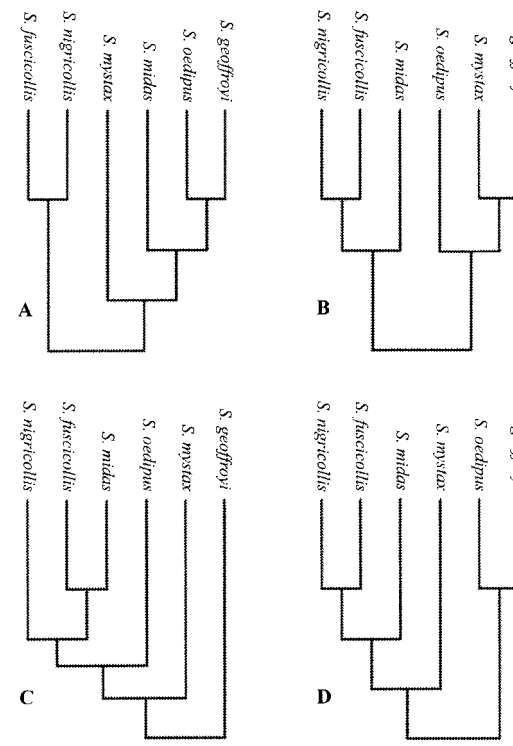

Fig. 3. Cluster diagrams representing hypothetical phylogenetic relationships among tamarin species, as reconstructed from four different sources. **A:** Mitochondrial sequence data (Cropp et al., 1999). **B:** Matrix correlation analysis (MxC). **C:** Correlations between the first principal components of the covariance matrices (PCI). **D:** Morphological distances between group means (D^2).

demonstrates that the patterns of variation and covariation between the tamarin species are, at least in part, consistent with their molecularly derived phylogenetic history (Cropp et al., 1999), as well as their relationships determined from studies of feeding behavior (Garber, 1992). Returning to the CPC analysis, the same patterns can be teased from the results even though the results themselves were not "significant." When the AIC was used to identify common structure, shared full principal component structure was observed between a number of species pairs. Interestingly, two of the species (*S. oedipus* and *S. geoffroyi*) distinguished from the others in the CPC analysis due to less structural similarity in principal component structure are among those found

to be most different using the matrix correlation method, and are sister species in Cropp et al. (1999).

CONCLUSIONS

Two methods for analyzing the degree of similarity between patterns of covariation produce very different objective results when applied to the task of evaluating differences in cranial-facial morphological variation patterns of six closely related species of New World monkeys. However, the strengths and shortcomings of matrix correlation and CPC methods appear complementary (not a surprising consequence, since one is grounded in evaluating similarity and the other in difference), making the use of both for analyzing the relationships between covariance matrices prudent. Such broader assessment of the tamarin covariation patterns shows that both methods can be simultaneously useful and correct; while the species are significantly different (CPC analysis), this difference does not preclude the possibility of moderate to high overall similarity (matrix correlation analysis), as well as allometric similarity (excluding *S. geoffroyi*) and similarity in patterns of morphological integration.

General patterns of mammalian cranial growth and development, such as disjunction of the neural and somatic growth systems, may result in common patterns of morphological integration across mammals— patterns which have been found in various species of primates and rats (Cheverud, 1982, 1995; Zelditch, 1988; Zelditch and Carmichael, 1989; Zelditch et al., 1990). The existence of a common pattern of morphological integration between the tamarin species is closely linked to the presence of common correlation patterns, so our detection of strong correlations between the tamarin species is not surprising.

However, the presence of discernible (and significant) differences in correlation/covariance structure at this species level of comparison cautions against uncritical extrapolation of constancy to other taxonomic levels. Others have found that the variation in correlation pattern in subspecific populations does not relate to phylogenetic or geographic relationships (Riska, 1985; Wagner, 1989), suggesting that at lower taxonomic levels the variation may be random and perhaps controlled by evolutionary processes such as genetic drift (Cheverud et al., 1989; Riska, 1985; Steppan, 1997b). Certainly changes in covariance and correlation patterns could be associated with major shifts in function, or with pronounced morphological divergence between organisms through evolutionary time. Similarity in the patterns of correlation/covariance was shown to relate somewhat to the phylogeny of the tamarin species being considered, although the meaning of this needs to be explored further. Conversely, this study does find a degree of correspondence between the covariation patterns of the tamarin species and their phylogenetic history, providing some evidence for directionality in the divergence of variance/covariance structure through evolutionary time at higher taxonomic levels. This can be interpreted as evidence that whether or not patterns of covariation behave predictably over macroevolutionary time, or even if they are similar, the assumption that they are constant is open to question.

ACKNOWLEDGMENTS

We thank those who provided generous help and access to the tamarin skeletal material: B. Patterson, S. Stanley, and L. Heaney (Field Museum of Natural History, Chicago), R. Thorington and R. Chapman (National Museum of Natural History), S. Tardiff and F. Smith (University of Tennessee), P.E. Vanzolini (Museu de Zoologia, University of Sao Paolo), R. MacPhee (American Museum of Natural History), and the staff at the British Museum of Natural History. Special thanks are due to S. Cropp for her contributions to the collection and analysis of the tamarin data, to T. Vaughan for his comments on earlier drafts of the manuscript, to P. Phillips for his help with interpreting the results of the CPC analyses, and to two anonymous reviewers whose remarks greatly improved the final product.

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